REMARKS/ARGUMENTS

Reconsideration and withdrawal of the rejections of the present application are respectfully requested in view of the amendments to the claims and remarks presented herewith, which place the application into condition for allowance.

Status of the Claims and Formal Matters

Claims 1-44 are currently pending in this application. Claims 16-21, 23, and 35-44 were withdrawn from consideration as allegedly being directed to a non-elected invention. Applicants hereby assert the right to reclaim withdrawn or cancelled subject matter in co-pending applications. By this paper, Claims 4 and 5 were cancelled and Claims 1, 3, 22, and 34 have been amended, without prejudice and solely to advance prosecution in accordance with the U.S. Patent and Trademark Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). No new matter has been introduced by these amendments. Support for the amended recitations can be found throughout the specification as originally filed, particularly at paragraphs [0026] to [0029] of the instant application published as U.S. Patent Application No. 2005/0079510.

These amendments are not made for purposes of patentability within the meaning of §§101, 102, 103, or 112. Rather, these amendments are made for clarity and to round out the scope of protection to which Applicants are entitled.

Priority Claim

The Office Action contends that the disclosure of the priority document U.S. Provisional Application Ser. No. 60/443,471, filed January 29, 2003 fails to provide adequate support or enablement in the manner provided by the provisions of 35 U.S.C. §112, 1st paragraph for one or more claims of the present application. The Office Action alleges that a separate prior application, U.S. Provisional Application Ser. No. 60/476,504, filed June 6, 2003, does provide adequate support or enablement for the instant application and will be used as the priority date for purposes of examination. Applicants respectfully disagree and request reconsideration of the priority claim for reasons provided herein.

The instant application claims priority to a number of provisional applications that when considered separately or in combination, provides reasonable enablement for the methods of the present invention. These priority documents represent a continuum of rigorous and inventive

research that began with the concept of "double-ended sequencing," and which was optimized to arrive at the presently claimed invention of "bead nucleic acid amplification." In particular, Applicants respectfully direct the Examiner to U.S. Provisional Application Ser. No. 60/465,071, filed on April 23, 2003, the contents of which provide ample support for bead emulsion nucleic acid amplification. For example, see pages 46-48 of 60/465,071, which provide in Specific Aim 1 that nucleic acids of interest can be captured in solid-phase to beads, which allow for clonal amplification of the nucleic acid on a single bead in a single well. Specific Aim 2 discloses that amplification of DNA can be achieved in oil and surfactant-based emulsions to encapsulate capture beads which have the nucleic acid of interest bound thereto.

The inventive concept of bead emulsion nucleic acid amplification performed under conditions where one primer is present in substantially greater concentrations than the other primer was clearly contemplated in 60/465,071 (see, for example, page 46, Specific Aim 2). Where amplification of a single-stranded DNA molecule is desired, it is advantageous to modify the concentrations of one of the two primers in the reaction such that one strand is preferentially amplified over another. With this type of asymmetric PCR as the goal, several different approaches were used, including, for example, modifying the primer concentrations or, as described in U.S. Provisional Application Ser. No. 60/443,471 (filed January 29, 2003), protection of one primer species by derivatization with chemical moieties, and their subsequent deprotection after the first primer sequence has been elongated and the desired single-stranded species is sequenced. In this respect, the concept of preferentially amplifying one nucleic acid strand over another was clearly contemplated by the present inventors as early as January 29, 2003.

For at least all of these reasons, Applicants respectfully request that the Examiner consider the earliest effective filing date of the present application to be January 29, 2003, or alternatively, April 23, 2003, not June 6, 2003.

Rejections under 35 U.S.C. §102(a)

Claims 1-13, 22, 24, 25, and 30-33 were rejected under 35 U.S.C. §102(a) as allegedly being anticipated by Griffiths (U.S. Patent Application No. 2002/0119459; now U.S. Patent No. 6,808,882). Griffiths allegedly teaches a method for amplifying one or more nucleic acids by forming a water-in-oil emulsion to create a plurality of aqueous microreactors, wherein at least

one of the microreactors comprise a single nucleic acid template, a bead capable of binding to the nucleic acid, and amplification reaction solution containing reagents necessary to perform nucleic acid amplification; amplifying the nucleic acids in the microreactors to form amplified copies of said nucleic acids; and binding the amplified copies to the beads in the microreactors. Applicants respectfully traverse this rejection in view of the amendments and remarks presented herewith.

Griffiths relates to methods of isolating one or more genetic elements encoding a gene product having a desired activity, the expression of which results, directly or indirectly, in the modification of an optical property of a genetic element encoding the gene product, comprising the steps of compartmentalizing genetic elements into microcapsules, expressing the genetic elements to produce their respective gene products within the microcapsules, and sorting the genetic elements according to changes in their optical properties. Notably, Griffiths does not teach or disclose methods of nucleic acid amplification in a water-in-oil emulsion that comprises a plurality of molecules of a first primer species and a second primer species, wherein a concentration of the second primer species is substantially greater than that of the first primer species in the reaction solution. Further, Griffiths fails to teach or disclose amplification reactions using the first and second primer species to form amplified copies of a complementary nucleic acid, in which substantially all of the molecules of the first primer species in the reaction solution are depleted, and where the amplified copies are captured to beads using the first primer species. Advantageously, this immobilizes a high concentration of the amplification product on the bead, making it useful for other techniques such as, for example, pyrosequencing methods as described in paragraph [0030] of the instant specification published as U.S. Patent Application No. 2005/0079510.

To serve as an anticipating reference under §102, the reference must enable that which it is asserted to anticipate. *Elan Pharmaceuticals, Inc. v. Mayo Foundation for Medical Education & Research* 346 F.3d 1051, 68 U.S.P.Q.2d 1373 (Fed. Cir. 2003). Applicants respectfully contend that in view of the aforementioned deficiencies, namely a lack of disclosure in Griffiths relating to amplification reactions comprising a first and a second primer species, the concentration of the second primer species being substantially greater than that of the first primer species, amplification of a nucleic acid template under conditions where substantially all of the molecules of the first primer species in the reaction solution are depleted, and capturing the amplified copies onto beads using the first primer species, Griffiths is unavailable as a reference

under §102. Consequently, reconsideration and withdrawal of the §102(a) rejection over Griffiths are respectfully requested.

Rejections under 35 U.S.C. §103(a)

Claim 14 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths. The Office Action contends that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use emulsion droplets of larger sizes such as in the range of 50 μm as used by the applicant or in the range of 10 μm as used by Griffiths, since these differences in emulsion droplet size allegedly would not be expected to greatly alter the conditions for amplification. The Office Action further alleges that though the effective concentration of a single template DNA would be lower in the larger droplets, this would be offset by the larger absolute amounts of amplification reagents, such as nucleotides and primers. The Office Action further cites *In re Peterson* and *In re Aller* in support of the argument that a *prima facie* case of obviousness exists where the claimed range and the prior art range do not overlap but are close enough such that the skilled artisan would have expected them to have the same properties, and that it is not inventive to discover the optimum or workable ranges by routine experimentation. *In re Peterson* 65 USPQ2d 1379, 1382 (Fed. Cir. 2003); *In re Aller* 105 USPQ 233 at 235. Applicants respectfully disagree and traverse this rejection.

Under §103(a), a *prima facie* case of obviousness is established by meeting three basic criteria. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art references must teach or suggest all the claim limitations. MPEP §2143.

Griffiths relates to, *inter alia*, methods for isolating one or more genetic elements encoding a gene product having a desired activity in the expression of such gene product which results in the modification of an optical property of a genetic element encoding the gene product. As discussed herein, Griffiths is notably silent as to methods of amplifying nucleic acids in a water-in-oil emulsion, wherein a single reaction solution comprises a first and a second primer species that are present in the reaction under conditions whereby the concentration of the second primer species is substantially greater than that of the first primer species in the reaction solution. Griffiths also fails to teach or suggest amplification of a nucleic acid template wherein

substantially all of the molecules of the first primer species are depleted, and where the amplified copies are captured to beads in the microreactor using the first primer species. In view of this deficiency, the teachings of Griffiths fail to establish the third requirement for an obviousness rejection under §103(a). Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection over Griffiths.

Claims 15 and 26-29 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Jurinke et al (U.S. Patent No. 6,303,309; hereinafter "Jurinke"). The Office Action contends that it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine the method of Griffiths for amplifying nucleic acids in a microcapsule such as a water-in-oil emulsion with that of Jurinke for purification of PCR products using solid supports such as magnetic or Sepharose beads, since the use of such beads allows further purification and extensive washing to remove all excess reaction components prior to final recovery of the final PCR product. The Office Action further alleges that an ordinary practitioner would have been motivated to use magnetic or Sepharose beads as taught by Jurinke for binding and purifying PCR or other amplification products generated in a microreactor since these beads have a large capacity and high affinity for such products, especially when using highly stable binding pairs such as biotin and streptavidin to form complexes of the amplification products on the beads. In view of the amendments to the claims and remarks presented herewith, Applicants respectfully traverse this rejection.

As discussed herein, Griffiths fails to teach or suggest the instantly claimed methods of amplifying nucleic acids in aqueous microreactors comprising a first and a second primer species, wherein the concentration of the second primer species is substantially greater than that of the first primer species, amplifying the nucleic acids such that substantially all of the molecules of the first primer species in the reaction are depleted, and capturing the amplified nucleic acid copies onto beads using the first primer species. Jurinke relates to methods of dissociating a complex comprising a biotin compound and a biotin-binding compound, which includes contacting the complex with an effective amount of an amine, under condition such that the complex is dissociated, thereby forming a biotin compound and a biotin-binding compound. Notably, however, Jurinke is silent as to methods of amplifying nucleic acids in aqueous microreactors containing a single reaction solution that comprises a first and a second primer species, wherein the concentration of the second primer species is substantially greater than the

first primer species. Jurinke is also silent as to amplification reactions wherein substantially all of the molecules of the first primer species in the reaction are depleted, and subsequently capturing the amplified nucleic acid copies to beads using the first primer species. Therefore, Jurinke fails to cure the defects of Griffiths. Griffiths and Jurinke, whether considered separately or in combination with each other, fail to establish a *prima facie* case of obviousness under §103(a) because Griffiths and Jurinke do not teach each and every limitation of the instant claims. Reconsideration and withdrawal of the §103(a) rejection over Griffiths in view of Jurinke are respectfully requested.

Claim 34 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Nakano et al (J. Biotech. (2003) 102: 117-124; hereinafter "Nakano"). The Office Action contends that it would have been *prima facie* obvious to the skilled artisan at the time the invention was made to combine the methods of Griffiths and Nakano for amplifying nucleic acids in a microcapsule such as a water-in-oil emulsion since the amplification of a plurality of products as taught by Nakano is allegedly easily adaptable to the methods of amplifying nucleic acids in a microcapsule. The Office Action further argues that the ordinary practitioner would allegedly have been motivated to use the system of Griffiths for amplifying multiple nucleic acid targets of different sizes in an emulsion containing a bead, since these can easily be purified simultaneously on the same bead and later separated by sizing methods or simply analyzed by gel electrophoresis. Applicants respectfully disagree and traverse this rejection.

Griffiths is silent as to methods of amplifying a nucleic acid in an aqueous microreactor comprising a single reaction solution having a first and a second primer species, wherein the second primer species is present in concentrations substantially greater than the first primer species in the reaction. Griffiths also fails to teach or suggest amplification of a nucleic acid template using the first and second primer species wherein substantially all of the molecules of the first primer species in the reaction solution are depleted, and subsequently capturing the amplified nucleic acid copies onto beads using the first primer species. Nakano, like Jurinke, fails to cure the deficiencies of Griffiths because Nakano is also silent as to the instantly claimed methods of amplifying nucleic acids in the presence of a first and a second primer species, the second primer species being present in concentrations substantially greater than the first primer species. Nakano also fails to teach or suggest amplification of the nucleic acid under conditions

where substantially all of the molecules of the first primer species in the reaction are depleted, and capturing the amplified nucleic acid copies to beads using the first primer species. Therefore, Griffiths and Nakano, considered together or separately, do not teach each and every element of the instant claims and cannot be used in support of an obviousness rejection under §103(a).

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection over Griffiths in view of Nakano.

CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion regarding this Response is desired, the Examiner is respectfully urged to contact the undersigned at the number given below, and is assured of full cooperation in progressing the application to allowance.

Respectfully submitted,

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